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Traversing worlds - Dispersal potential and ecological classification of *Speolepta leptogaster* (Winnertz, 1863) (Diptera, Mycetophilidae)

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Abstract

Speolepta leptogaster (Winnertz, 1863) is frequently occurring in European subterranean environments. As for most cave animals, studies addressing non-anatomical aspects are sparse. Here we present the first molecular study on *S. leptogaster*. We investigated the demographic structure (i.e. COI locus) of 69 specimens from 36 underground populations in Hesse (Central German Uplands) to get first insights into the species' dispersal ability.

In total, 14 haplotypes were revealed. Haplotype diversity was relatively high, whereas nucleotide diversity was low. Furthermore, a significant but low pattern of isolation-by-distance and (a) past population expansion event(s) were detected.

Our genetic results suggest a (good) active dispersal ability for *Speolepta leptogaster*. The occurrence of several surface records of adult specimens corroborates this hypothesis. We discuss the developmental stages of *S. leptogaster* in the context of the ecological classification system and regard the species as a eutroglophile. Evidence has been found to distinguish two larval types. A reconstructed life-cycle of the species is provided.

Keywords

Cave animal, ecotone, phylogeography, mobility, ecological versatility

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Introduction

Century and a half have passed since the mycetophilid *Speolepta leptogaster* (Winnertz, 1863) (Diptera, Mycetophilidae) has been described. The species is widely distributed throughout Europe and can complete its entire life-cycle in subterranean environments such as caves, mines or related underground habitats. Originally placed into the genus *Polylepta*, Edwards (1925) established the monotypic genus *Speolepta* in reference to its subterranean ecology and morphology. Due to its enigmatic ecology, obligate subterranean larvae spinning silk-nets and the short adult life spans, *S. leptogaster* received popular attention amongst entomologists (Schmitz 1912, Lengersdorf and Mannheims 1951, Matile 1962, Plachter 1981). As a tribute, the species was chosen as the Cave Animal of the Year 2013 in Germany. Despite all the scientific attention, research primarily focused on morphological investigations of the different developmental stages (egg, larval stages, pupa and imago) (Schmitz 1912, Lengersdorf and Mannheims 1951, Matile 1962, Plachter 1981), rather than on biological or ecological characteristics. As recently as 2012, two other *Speolepta* species have been described, thus increasing the number of known species in this genus up to three (Sevcik et al. 2012). In their taxonomic revision, Sevcik et al. (2012) pointed to neglected investigations concerning the species' dispersal ability. They suggested that "[a]lthough the European *S. leptogaster* normally breeds and completes its life-cycle entirely underground (Matile 1962), adults of both sexes are frequently found far away from caves. [...] This suggests good dispersal abilities where some gene flow may be retained between otherwise very isolated cave populations".

Here, we investigate the dispersal ability of *Speolepta leptogaster* by integrating population genetic data of specimens collected from underground sites in the Central German Uplands (Hesse). The study region was chosen since it was covered by permafrost during the Last Glacial Maximum (18,000 – 24,500 ybp) (Clark et al. 2009). A postglacial (re-)colonization of this area (inferring a certain level of mobility) rather than the survival in a central German glacial refugium is likely.

Methods

Material and morphological analyses

In total, 69 specimens of *Speolepta leptogaster* from 37 different caves, bunkers, wells, tunnels and cellars in Hesse (maximum of three per site) and one from a bunker in Poland were analyzed. The samples (Table 1) were collected and identified by members of the Hesse Federation for Cave and Karst Research (Germany) (Reiss et al. 2009) and stored in ethanol. In order to ensure good DNA preservation, all molecularly-processed samples were dated no later than six years old. Prior to DNA isolation, images of all specimens were taken with the camera "Moticam Model Moticam 5". Specimens

Table 1. Dataset of analyzed specimens of *Speolepta leptogaster* and locality information. LS: life stage (L: larva, P: pupa, I: imago); H: haplotype. The geographic coordinates are in Degrees (°), Minutes (') and Decimal seconds ("). The locality numbers resemble the numbers given to each natural region by the German Bundesamt für Naturschutz.

#	LS	Coordinates	Biotope	Locality numbers	H	NCBI
1a	L	50°10'13.80"N; 9°24'11.92"E	natural cave	141 Sandsteinspessart	9	KF624625
1b	L	50°10'13.80"N; 9°24'11.92"E	natural cave	141 Sandsteinspessart	1	KF624626
1c	L	50°10'13.80"N; 9°24'11.92"E	natural cave	141 Sandsteinspessart	4	KF624627
2a	L	50°15'49.43"N; 9°31'39.86"E	natural cave	141 Sandsteinspessart	1	KF624628
2b	L	50°15'49.43"N; 9°31'39.86"E	natural cave	141 Sandsteinspessart	1	KF624629
3a	I	50°4'55.99"N; 7°48'56.02"E	mine shaft	304 Westlicher Hintertaunus	1	KF624630
3b	I	50°4'55.99"N; 7°48'56.02"E	mine shaft	304 Westlicher Hintertaunus	1	KF624631
4a	P	50°5'34.37"N; 7°51'6.16"E	mine shaft	304 Westlicher Hintertaunus	8	KF624632
4b	L	50°5'34.37"N; 7°51'6.16"E	mine shaft	304 Westlicher Hintertaunus	8	KF624633
5a	L	50°6'36.32"N; 7°56'49.81"E	mine shaft	304 Westlicher Hintertaunus	2	KF624634
5b	L	50°6'36.32"N; 7°56'49.81"E	mine shaft	304 Westlicher Hintertaunus	2	KF624635
5c	L	50°6'36.32"N; 7°56'49.81"E	mine shaft	304 Westlicher Hintertaunus	2	KF624636
6	L	50°9'19.87"N; 8°4'53.98"E	mine shaft	304 Westlicher Hintertaunus	1	KF624637
7a	L	50°52'40.73"N; 8°28'11.28"E	mine shaft	320 Gladenbacher Bergland	7	KF624638
7b	L	50°52'40.73"N; 8°28'11.28"E	mine shaft	320 Gladenbacher Bergland	7	KF624639
8	L	50°53'21.05"N; 8°25'39.54"E	mine shaft	320 Gladenbacher Bergland	11	KF624640
9	L	50°52'23.23"N; 8°28'37.34"E	rock cellar	320 Gladenbacher Bergland	3	KF624641
10a	L	51°0'4.61"N; 8°35'41.53"E	mine shaft	332 Ostsauerländer Gebirgsrand	2	KF624642
10b	L	51°0'4.61"N; 8°35'41.53"E	mine shaft	332 Ostsauerländer Gebirgsrand	2	KF624643
10c	L	51°0'4.61"N; 8°35'41.53"E	mine shaft	332 Ostsauerländer Gebirgsrand	2	KF624644
11a	L	51°23'34.73"N; 8°41'28.79"E	mine shaft	332 Ostsauerländer Gebirgsrand	1	KF624645
11b	L	51°23'34.73"N; 8°41'28.79"E	mine shaft	332 Ostsauerländer Gebirgsrand	14	KF624646
11c	L	51°23'34.73"N; 8°41'28.79"E	mine shaft	332 Ostsauerländer Gebirgsrand	1	KF624647
12a	L	51°13'56.89"N; 8°54'4.32"E	natural cave	340 Waldecker Tafelland	2	KF624648
12b	L	51°13'56.89"N; 8°54'4.32"E	natural cave	340 Waldecker Tafelland	2	KF624649
13a	L	51°14'25.08"N; 8°54'9.40"E	mine shaft	340 Waldecker Tafelland	1	KF624650
13b	L	51°14'25.08"N; 8°54'9.40"E	mine shaft	340 Waldecker Tafelland	1	KF624651
14	L	51°18'55.58"N; 9°24'26.86"E	undercroft	342 Habichtswälder Bergland	1	KF624652
15a	L	51°7'41.70"N; 8°59'0.17"E	spring	344 Kellerwald	1	KF624653
15b	L	51°7'41.70"N; 8°59'0.17"E	spring	344 Kellerwald	1	KF624654
16	L	51°7'32.66"N; 8°59'33.47"E	spring	344 Kellerwald	1	KF624655
17	L	50°30'3.56"N; 9°7'25.43"E	rock cellar	350 Unterer Vogelsberg	1	KF624656
18a	L	50°31'0.59"N; 9°32'3.73"E	mine shaft	350 Unterer Vogelsberg	1	KF624657
18b	L	50°31'0.59"N; 9°32'3.73"E	mine shaft	350 Unterer Vogelsberg	1	KF624658
19	I	50°27'0.29"N; 9°49'15.92"E	rock cellar	353 Vorder- und Kuppenrhön	2	KF624659
20	L	50°34'21.18"N; 9°57'38.63"E	rock cellar	353 Vorder- und Kuppenrhön	12	KF624660
21	L	50°35'23.96"N; 9°59'54.13"E	rock cellar	353 Vorder- und Kuppenrhön	1	KF624661
22a	P	50°35'57.73"N; 9°59'59.60"E	culvert	353 Vorder- und Kuppenrhön	1	KF624662
22b	P	50°35'57.73"N; 9°59'59.60"E	culvert	353 Vorder- und Kuppenrhön	1	KF624663

#	LS	Coordinates	Biotope	Locality numbers	H	NCBI
23a	L	50°30'30.64"N; 9°55'48.65"E	mine shaft	354 Hohe Rhön	1	KF624664
23b	L	50°30'30.64"N; 9°55'48.65"E	mine shaft	354 Hohe Rhön	6	KF624665
23c	L	50°30'30.64"N; 9°55'48.65"E	mine shaft	354 Hohe Rhön	6	KF624666
24a	L	50°28'7.25"N; 9°57'45.40"E	spring	354 Hohe Rhön	1	KF624667
24b	L	50°28'7.25"N; 9°57'45.40"E	spring	354 Hohe Rhön	1	KF624668
25a	L	50°52'35.18"N; 9°42'27.40"E	brick-built cellar	355 Fulda-Haune-Tafelland	1	KF624669
25b	L	50°52'35.18"N; 9°42'27.40"E	brick-built cellar	355 Fulda-Haune-Tafelland	1	KF624670
26a	L	50°53'4.56"N; 9°43'25.10"E	rock cellar	355 Fulda-Haune-Tafelland	1	KF624671
26b	L	50°53'4.56"N; 9°43'25.10"E	rock cellar	355 Fulda-Haune-Tafelland	1	KF624672
27a	L	50°51'35.21"N; 9°45'19.94"E	mine shaft	355 Fulda-Haune-Tafelland	1	KF624673
27b	L	50°51'35.21"N; 9°45'19.94"E	mine shaft	355 Fulda-Haune-Tafelland	13	KF624674
27c	L	50°51'35.21"N; 9°45'19.94"E	mine shaft	355 Fulda-Haune-Tafelland	1	KF624675
28a	L	51°7'34.36"N; 9°47'35.70"E	brick-built tunnel	357 Fulda-Werra-Bergland	1	KF624676
28b	L	51°7'34.36"N; 9°47'35.70"E	brick-built tunnel	357 Fulda-Werra-Bergland	10	KF624677
29	L	51°12'26.24"N; 9°52'16.82"E	mine shaft	357 Fulda-Werra-Bergland	1	KF624678
30	L	51°0'43.13"N; 9°55'45.73"E	mine shaft	357 Fulda-Werra-Bergland	1	KF624679
31a	L	51°0'24.88"N; 9°57'44.68"E	mine shaft	357 Fulda-Werra-Bergland	5	KF624680
31b	L	51°0'24.88"N; 9°57'44.68"E	mine shaft	357 Fulda-Werra-Bergland	5	KF624681
31c	L	51°0'24.88"N; 9°57'44.68"E	mine shaft	357 Fulda-Werra-Bergland	5	KF624682
32a	L	51°13'29.10"N; 9°57'8.68"E	mine shaft	358 Unteres Werratal	1	KF624683
32b	L	51°13'29.10"N; 9°57'8.68"E	mine shaft	358 Unteres Werratal	1	KF624684
33a	L	51°13'27.05"N; 9°57'11.74"E	touristic mine	358 Unteres Werratal	2	KF624685
33b	L	51°13'27.05"N; 9°57'11.74"E	touristic mine	358 Unteres Werratal	2	KF624686
34a	L	51°10'45.44"N; 10°4'15.28"E	mine shaft	358 Unteres Werratal	1	KF624687
34b	L	51°10'45.44"N; 10°4'15.28"E	mine shaft	358 Unteres Werratal	1	KF624688
35	L	51°31'8.11"N; 9°22'39.43"E	bunker complex	361 Oberwälder Land	1	KF624689
36a	L	51°31'8.11"N; 9°22'39.43"E	bunker complex	361 Oberwälder Land	1	KF624690
36b	L	51°31'8.11"N; 9°22'39.43"E	bunker complex	361 Oberwälder Land	1	KF624691
36c	L	51°31'8.11"N; 9°22'39.43"E	bunker complex	361 Oberwälder Land	1	KF624692
37	I	52°24'0"N; 15°31'59.99"E	bunker complex	Nietoperek (Poland)	8	KF624693
Σ 69						

were measured using the measuring function of the image-capturing program "Motic Images Plus 2.0". The bodies of the specimens were examined to determine potential differences in phenotype, which could correspond to different haplotypes.

Molecular analyses

A small piece of the posterior end of the body (approx. one sixth of the total animal) was used for larval samples. In the case of pupae and imagines, a larger portion of the abdomen (approx. ¼) was macerated. DNA isolation was performed according to the instructions of the DNEasy Blood & Tissue Kit (Qiagen Sample and Assay Technologies, Hilden, Deutschland) for the column purification of animal tissue.

A 662 bp fragment of the Cytochrome C Oxidase subunit 1 (COI) gene was amplified using the primers C1-J-2195 5'-TTGATTTTTTGGTCACCCTGAAGT-3' and TL2-N-3014 5'-TCCAATGCACTAATCTGCCATATTA-3' established by Simon et al. (1994). The PCR reactions were executed in a Peqlab Primus Advanced 96 thermo cycler. Each PCR-mix (25 µL) contained 2.5 µL PCR-buffer (10×, without $MgCl_2$), 2.0 µL $MgCl_2$ (100 mM), 0.3 µL dNTPs (20 mM), 1.0 µL of each primer, 0.3 µL Taq-DNA-Polymerase, 1.5 µL BSA (bovine serum albumin, 10 mg/mL), 11.4 µL ddH_2O and 5.0 µL template DNA. PCR conditions were as follows: initial denaturation step 1 min 94 °C, 30 cycles of denaturation (30 sec, 94 °C), annealing (30 sec, 43.7 °C) and elongation (30 sec, 72 °C) and a final elongation step with 7 min at 72 °C. PCR products were bi-directionally sequenced using the Sanger chain termination method (Sanger et al. 1977). Sequencing service was provided by the Laboratory Centre of the Biodiversity and Climate Research Centre (Frankfurt am Main, Germany) with their own sequencing protocols. Editing and assembly of the forward and reverse sequences were done using Geneious v5.4.6. The sequences were manually trimmed to a length of 659 bp to fit the shortest sequence retained. Sequence alignment was performed using the MAFFT v6.814b plug-in for Geneious under default settings and the automatic algorithm option.

Population genetic analyses

The software DnaSP v5 (Librado and Rozas 2009) was used to calculate the number of haplotypes, the total haplotype and nucleotide diversity and to perform neutrality tests. Haplotype diversity (Hd) can result in values between 0 and 1. A value of Hd = 1 implies that two randomly picked samples will always demonstrate two different haplotypes (= 100% diversity). The nucleotide diversity (π) illustrates the average genetic distance between two sequences estimated as an average for all sequences. The value can range from 0 (no changes in all sequences) to a theoretical 1 (every base is replaced). The calculation for Fu's Fs (Fu 1997) and Tajima's D (Tajima 1989) tests can be positive, negative or have a zero value. A significant positive value for Tajima's D may point to over dominant selection or a population bottleneck event. Significant negative values point to a population expansion event or purifying selection. A value of zero or non-significant values cannot reject the neutral model of molecular evolution. Fu's Fs shares the same characteristics but adds the support for genetic hitchhiking with a negative value. Standard settings were used and the sequences were trimmed to the same length. The program TCS 1.21 (Clement et

al. 2000) was used to reconstruct a haplotype network by Statistical Parsimony (Templeton et al. 1992). Networks were created by a 95% connection probability.

A Mantel-test (Mantel 1967) was used to test for correlation between geographic distance vs. genetic distance. The geographic distance matrix was calculated with the Geographic Distance Matrix Generator 1.2.3 (Ersts 2014) using a WGS84 spheroid and geodesic distances in km. Genetic distances were calculated as p-distances using the software MEGA 5.2 (Tamura et al. 2011) and the pairwise deletion option. The Mantel-test was performed in XLSTAT 2013 (Addinsoft) using the linear Pearson product-moment correlation coefficient (r) and 10,000 permutations. A value of r = 0 implies no linear correlation, whereas a minimal / maximal value of -1 / +1 indicates total negative / positive correlation.

Results

Demographic structure of *S. leptogaster* in Hesse

No gaps were present in the COI-alignment. In total, 14 haplotypes (H1-H14) were revealed. Haplotype diversity was high with a value of Hd = 0.7017, whereas nucleotide diversity was low with π = 0.00165 (Table 2). The haplotype network (Figure 1) indicated a highly frequent haplotype, H1, which consisted of 39 samples (57%) and a second relatively frequent haplotype, H2, which comprised 11 samples (16%). Eight haplotypes were singletons (H3, H4, H9-H14), two consisted of two (H6 and H7) and two of three samples (H5 and H8). There were only four haplotypes (H4, H8, H9 and H11) which were not directly connected to the most frequent haplotype H1.

When the different haplotypes are placed in a geographical context (Figure 2), H1 can be revealed throughout the study region of Hesse. Haplotype 2 demonstrates a similar distribution except in the central underground sites and the most northern parts of Hesse. Haplotype 8 occurs once in southern Hesse and in the sampling site in Poland. All other haplotypes have been found within only a single locality. At a few localities several haplotypes co-occurred: In north-western Hesse H14+H1 and H2+H1; in north-eastern Hesse H9+H1, H2+H1 and H13+H1 and in south-eastern Hesse, H10+H6+H1.

Table 2. Overview of statistical values. Estimates are given for neutrality tests (Fu's Fs, Tajima's D), haplotype (Hd), nucletide diversity (π) and Pearson's r in a Mantel-test (geographic distance vs. genetic distance).

estimates	value	p-value
Fu's Fs	-10.28	$p < 0.001$
Tajima's D	-1.89	$p = 0.004$
Hd	0.7017	
π	0.00165	
r	0.17	$p < 0.0001$

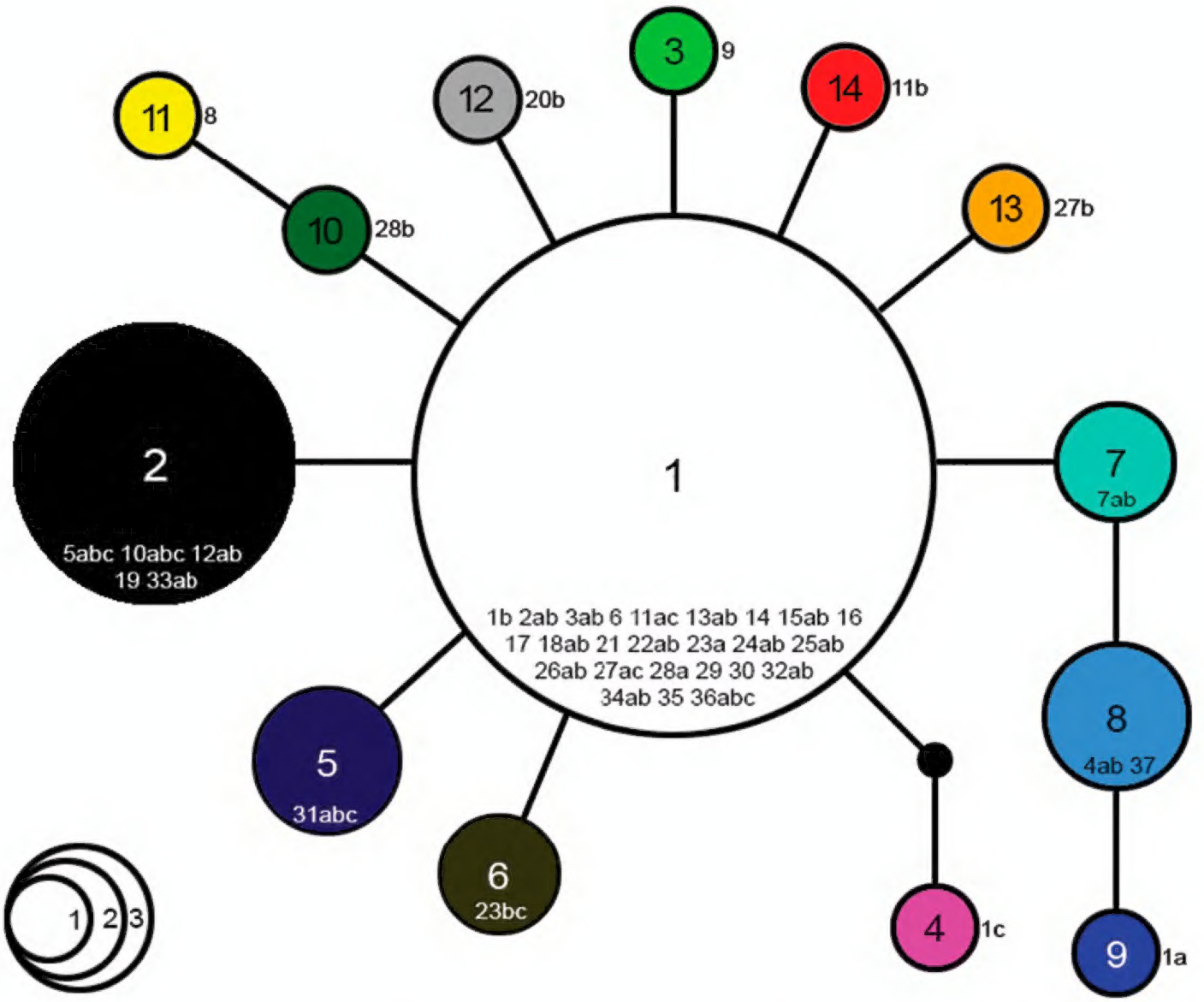

Figure 1. CO1 haplotype network for *Speolepta leptogaster*. Haplotypes are numbered in sequence with their volume proportional to their frequency in the total dataset. Lines interconnecting the haplotypes illustrate the mutational course and the number of mutational steps between them. Numbers with letters within or alongside circles refer to Table 1.

The results of both neutrality tests were significantly negative ($p < 0.01$) and point to (a) past population expansion event(s) (Table 2). In our dataset, 28.5% of all mutations (4/14) were non-synonymous leading to a change of the respective amino acid.

The Mantel-test ($r = 0.17$, $p < 0.0001$) reveals a significant positive low correlation between genetic distance and geographic distance (Table 2).

Discussion

Dispersal ability

The region of the Central German Uplands (including our target area of Hesse) was covered with permafrost during the Last Glacial Maximum (18,000 – 24,500 years ago) (Clark et al. 2009). It is widely accepted, that ecologically diverse species were able to (re-)colonize this area when the climatic conditions became more suitable after

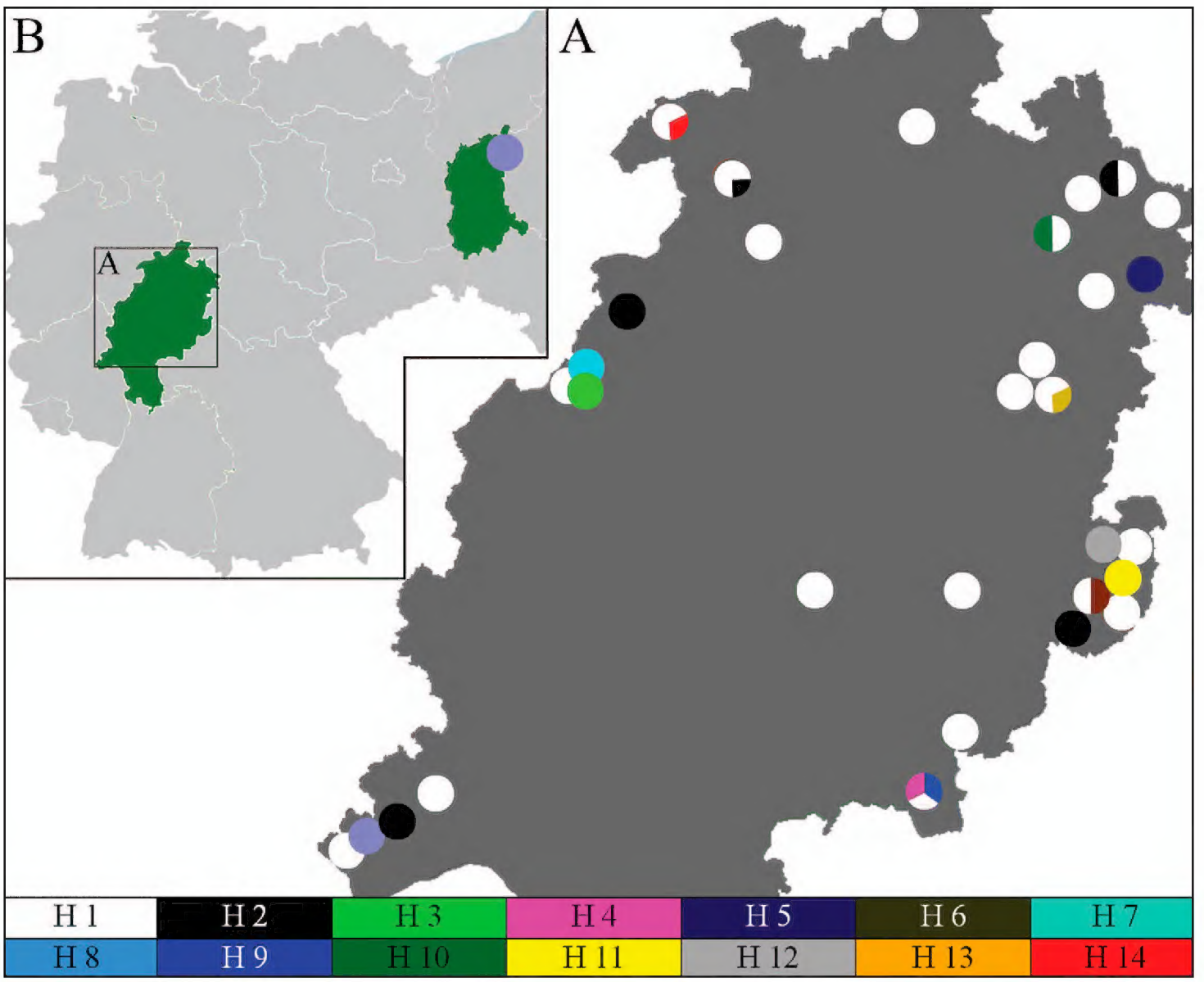

Figure 2. Spatial pattern of haplotypes of *Speolepta leptogaster* in Hesse**.** The haplotype (H) distribution of *S. leptogaster* within Hesse (**A**) with a comparison group in Poland (**B**) is depicted as a circle for every underground locality with colored sections for the different haplotypes. To be depicted in a reasonable manner, multiple localities were reduced to one circle if they were situated nearby (up to 4 km) and had the same color.

the ice shields had retracted northward again (Taberlet et al. 1998, Hewitt 1999, Petit et al. 2002). Our genetic analyses are in agreement with a similar postglacial scenario for *Speolepta leptogaster.* The recent demographic structure of this species in Hesse is best explained by (a) past population expansion event(s). Most probably and after population bottlenecks, the low-frequency and most often cave endemic haplotypes have evolved from the high-frequency central haplotypes already present in the ancestral gene pool of the species (i.e. the founder effect). The significant but weak pattern of isolation-by-distance can be interpreted in terms of a certain level of connectivity between populations, which may be explained by dispersal of adult specimens. An interesting finding is the identity of haplotypes found in Poland and southern Hesse. Yet, a pan-European sampling is needed to address any further hypotheses (e.g. passive anthropogenic transportation vs. active surface-dispersal).

In general, our results imply good dispersal ability for *Speolepta leptogaster*. This is further supported by surface records of adult specimens compiled from the literature

Box 1. Original description of *Speolepta leptogaster* (Winnertz, 1863). Translated from German. Originally this species has been described under the name of *Polylepta leptogaster*.

The habitus is very similar to the *Bolitophila*. Body color brown. Mouth-rim slightly pulled forward and garlanded with hairs. The filamentous antennae are about 1.33 times as long as head and thorax together. The flagellum links are 3 to 4 times as long as broad. The haltere is whitish with a black-brown tip. The abdomen is very slim and cylinder-shaped, about 5 to 6 times as long as the very short thorax and constricted at the base. The coxa and femur yellow, tibia more brownish, tarsus light brown. The feet of the front legs are 2.33 times as long as the tibia, the tibia slightly shorter than the metatarsus (9 : 9 ¾) with lanceolate basis. Wings slightly greyish nearly colorless; the subcosta proceeding over the cubitus up to the tip of the wing, the supporting vein broken off in front of the lateral vein, the marginal lateral vein pulled far back, the discal cell trapezoid-shaped, 1.5–2 times as long as broad, the style of the upper fork about half the length of the upper prong, the basis of the rear cell under the middle of the wing on the far side of the discal cell, the axilla vein not sturdy, broken off on the opposite side of the rear cell. I only captured a female of this very rare species once in august in a swampy, forested area. A second female is located at the Royal Museum in Leyden, which differs from mine in the way that the discal cell, which is 1.5 times as long as broad in my specimen, is 2 times as long as broad. Apart from that, they completely matched.

(Table 3). Although all developmental stages can be found in caves throughout the year, there are more findings of surface imagines in summer than in winter. Whether this is due to the biology of *S. leptogaster* or to the smaller number of traps being laid out in winter cannot be determined.

Ecological classification

Animals associated with underground habitats can be classified into different categories. Those classifications (i.e. ecological, behavioral, morphological) have been under constant change and revision. Multiple authors created new categories as well as split up old ones (Shiner 1854, Racovitza 1907, Vandel 1965, Hamilton-Smith 1971, Sket 2008). However, the main ecological categories to which we refer here have remained more or less unchanged: eutrogloxenes (normally not living in caves), subtroglophiles (partially living in caves, but without permanent populations), eutroglophiles (able to complete several underground generations) and eutroglobionts (solely living in caves). As a result of this ecological continuum of cave-association, i.e. from "only found by chance" to "being obligate subterranean", probable cave-associated (e.g. morphological and behavioral) adaptations are multifarious.

Since the biological diversity of subterranean species and ecology of subterranean habitats is high (Moseley 2008), every categorical and thus limited ecological classification system will elicit problems in classifying all cave organisms. This is particularly challenged by a "species from both worlds" living in a transitional environment or ecotone, as which caves must be considered (Moseley 2008). The situation is even more complicated

Table 3. Surface records of *Speolepta leptogaster*. N: number of specimens found, f: female, m: male.

Region	Date of collection	Habitat/collection	N	Reference
		swampy forest area	1 f	Winnertz (1863)
Germany, Birgsau, südlich von Oberstdorf, im Stillachtal	18–27 Sept. 1975	light trap	1 m	Plassmann (1977), first description *S. dissona*
Germany, Saxon Switzerland 1.5 km SE of Ober-vogelgesang	10–21 May 1997	deciduous forest, Malaise trap	1 f, 1 m	U. Kallweit (unpublished)
S-Germany, Grenzach	11 May 2008		1 f	B. Rulik (unpublished)
Germany, Harz mountains	26 Oct. 2004	natural mature spruce forest, yellow pan trap	2 m	U. Kallweit (unpublished)
Germany, Harz mountains	25 June 2004	natural mature spruce forest, Malaise trap	1 m	U. Kallweit (unpublished)
Germany, Hesse, Fulda, Mittelbergquelle	10 Oct. 2004	hand collection	1 f	Zaenker (2008)
Germany, Hesse, Auersbergquelle 21	18 Sept. 2009	sweep net	1 f	Zaenker unpublished
Germany, Hesse, Lützeler Sang-Quelle 2	25 Oct. 2007	sweep net	1 m	Zaenker (2008)
Norway, Kvinnherad, Rosendal, riverside at Avlsgården, Baroniet	11–15 May 1990	Malaise trap	1 f, 2 m	Ševčík et al. (2012)
Norway, Bergen, Haukeland	9 May–28 June 1991	Malaise trap	3 m	Ševčík et al. (2012)
Norway, Bømlo, Vorland, Langevåg	11 Feb 2002–12 Feb 2003	Malaise trap	7 f, 5 m	Ševčík et al. (2012)
Norway, Etne, Skånevik skyttarbane	3 Sept. 2009	sweep net	1 f, 1 m	Ševčík et al. (2012)
Norway, Fjell, Vindenes	5 Sept. 1978	light trap	1 f	Ševčík et al. (2012)
Norway, Os, Raudli	23–30 May 1991		1 f, 1 m	Ševčík et al. (2012)
Norway	June–Sept. 1991	Malaise trap	17 f, 11 m	Ševčík et al. (2012)
Norway, Os, Sæleli	20–27 June 1991	Malaise trap	1 m	Ševčík et al. (2012)
Norway, Øygarden, Dalsvann, Alvøy	5 June 1987	light trap	1 f	Ševčík et al. (2012)
Norway, Sveio, Førde, Solheimshaugen	3–10 June 1991	Malaise trap	2 f	Ševčík et al. (2012)
Norway, Sveio, Førde, Solheimshaugen	15 June 1991	sweep net	1 f, 1 m	Ševčík et al. 2012

Region	Date of collection	Habitat/collection	N	Reference
Norway, Sunndal, Jordalsgrenda, Jordalsøra, Hamrene	31 Mai–13 Jul. & 26 Aug.–6 Sept. 2004	Malaise trap	9 f, 14 m	Ševčík et al. (2012)
Norway, Sunndal, Jordalsgrenda, Jordalsøra, Hamrene	14 June–3 Jul. & 12–25 Aug. 2005	window trap	1 f, 1 m	Ševčík et al. (2012)
Norway, Aurland, Vassbygdvatnet (lower end of lake)	4 Aug. 1969	sweep net	1 f	Ševčík et al. (2012)
Norway, Porsgrunn, Hitterødbekken	13 June–11 Jul. 1988	Malaise trap	3 f	Ševčík et al. (2012)
Sweden, Jokkmokk, Kaltisbäcken 1km NNE Messaure	19–30 June 1968	air suction trap	1 f, 3 m	Ševčík et al. (2012)
Sweden, Lund, Høje Å (stream) at Värpinge	23–28 May 2004	yellow pan trap	1 m	Ševčík et al. (2012)
Sweden, Klippan, Skäralid NR (ravine with stream)	26 Sept. 1983	sweep net	1 m	Ševčík et al. (2012)
Sweden, Högsby kommun, Getebro	29 Jul–31 Aug. 2004	Malaise trap	1 f	Ševčík et al. (2012)
Faroe Islands, Streymoy, Kvivik	13–17 Jul. 1990	Malaise trap	2 m	Ševčík et al. (2012)
Czech Republic, Bohemia, Jizerské Hory Mts, Jedlový důl	6–28 Jul. & 1–22 Sept. 2005	Malaise trap	3 m	Ševčík et al. (2012)
Czech Republic, Bohemia, Mt. Poledník	29 Aug. -5 Okt. 2004	Malaise trap	1 m	Ševčík et al. (2012)
Czech Republic, Bohemia, Krkonoše Mts., Bílé Labe	16–30 Aug. 2007	Malaise trap	1 m	Ševčík et al. (2012)
Czech Republic, Bohemia, Labský důl	24–27 Jul. 2006	Malaise trap	1 m	Ševčík et al. (2012)
Czech Republic, Moravia & Silesia, Hrubý Jeseník Mts, Velká kotlina	9–26 June 2006	Malaise trap	1 m	Ševčík et al. (2012)
Czech Republic, Moravia & Silesia, Rejvíz	20 May–1 Jul. 2005	peat-bog, Malaise trap	1 f	Ševčík et al. (2012)
Slovakia, Pol'ana Mts., Hrončecký grún	7 May–4 Jul. 2006	yellow pan trap	1 m	Ševčík et al. (2012)
Slovakia, Kyslinky–Pod Dudášom	15 June 2009		1 m	Ševčík et al. (2012)
Slovakia, Predná Pol'ana Mt.–Bystré waterfall	17 June 2009		1 m	Ševčík et al. (2012)
Slovakia, Spády waterfall	18 June & 4 Jul. 2009		2 m	Ševčík et al. (2012)
			[52 f, 64 m]	

by the fact that *Speolepta leptogaster* belongs to the holometabolic insects with different developmental stages demonstrating different ecological requirements. This species is thus a prime example that an ecological classification system for cave-dwelling *species,* based on four categories may be too ambiguous particularly in cases where different *life stages* may behave ecologically distinct. Here we discuss the species three effective life stages, including the larva, pupa and imago in respect to the ecological classification system. We characterize the single developmental stages and potentially the whole species.

The larvae hatch and live solely within subterranean habitats reaching from microcavities to macrocaverns (or caves). They are incapable of surviving on the surface, which is related to their preference for a highly water-saturated atmosphere and adaptation to oxygen respiration. They lack a trachea system but are able to respire oxygen through a very thin cuticula spanning the entire body surface (Schmitz 1912). Author's observations (SZ and AW) point to a behavior of avoiding strong air currents. Since the pupal stage is immobile and directly succeeds the larval developmental stage, it can only occur in the same habitat. The imago however, possesses elongated legs (sometimes regarded as a troglomorphy) (Schönborn 2003) and demonstrates a sluggish flight. At the same time, imagines do not feed and probably only survive a few days to weeks, thus complicating the inference of their ability to survive outside the subterranean habitat. However, several surface records of imagines (Table 3) and sightings of larvae in the transition and entrance zones of underground sites (Zaenker 2008, Weber 2012) are known. Furthermore, it seems that adult specimens travel between caves during the night.

Conclusively, not all developmental stages are capable of surviving outside the subterranean zone. Thus, the completion of a life-cycle aboveground will be incomplete – eliminating the eutrogloxenes as a potential ecological category for *Speolepta leptogaster*. Due to the short life-span of adults rarely leaving the subterranean zone, the complete life-cycle (or a very large proportion) is within the subterranean environment – leaving the eutroglophiles and eutroglobionts. From an evolutionary point of view and for long-term survival, *S. leptogaster* should be classified as a eutroglophile, i.e. being able to complete several underground generations but having the ability of surface dispersal. But pinpointing *S. leptogaster* to a single ecological category clearly underestimates the ecological versatility of the species.

Potential life-cycle

Even after 150 years, some facts about the biology of *Speolepta leptogaster* are still unknown. Specifically, these include the number of larval stages and the durations of the different life stages. Although Schmitz (1912) claimed that he had successfully bred *S. leptogaster* in captivity, he provided no information about the duration of any of the life stages. Still, a comparison with related Mycetophilidae (including the ecologically-similar keroplatid *Arachnocampa luminosa* (Skuse, 1890)) (Baker 2010, Li et al. 2011) may enable a vague picture of the potential life-cycle and the life-spans of the different

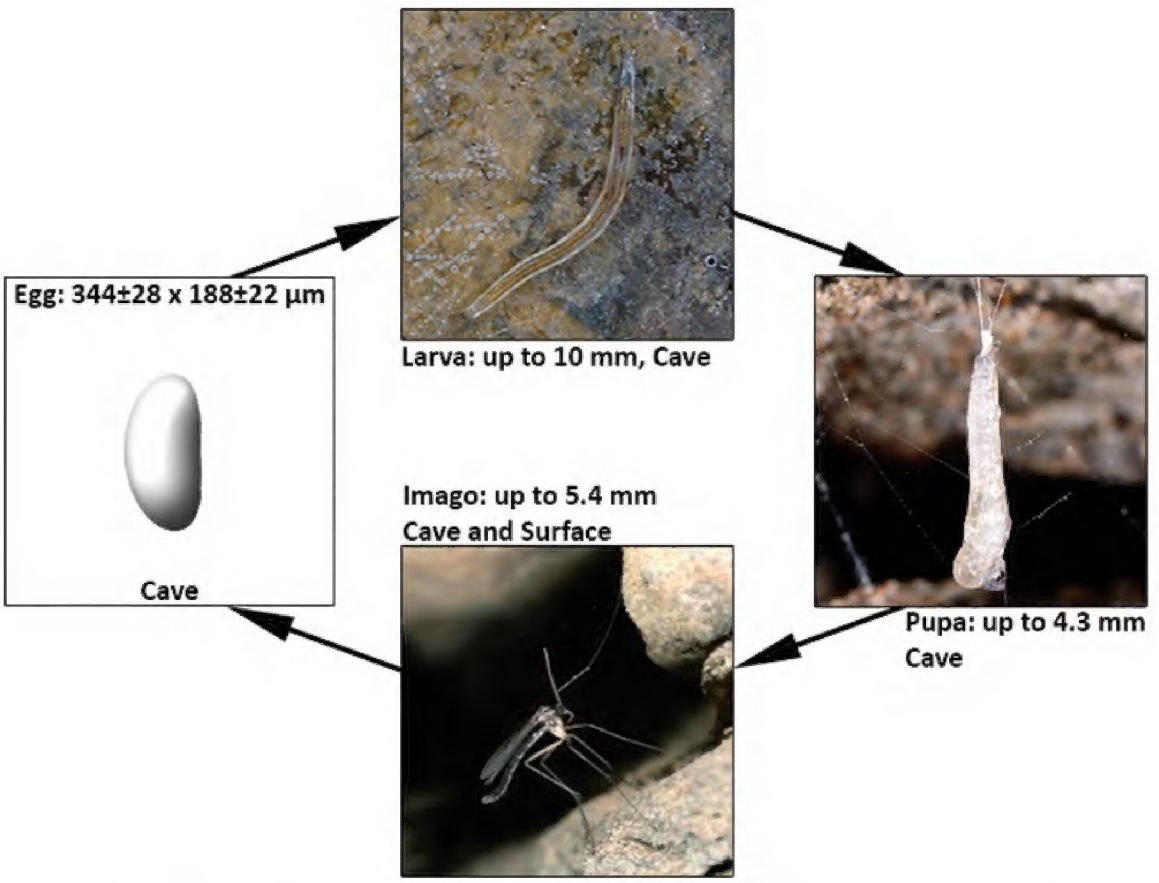

Figure 3. Potential life-cycle of *Speolepta leptogaster*; egg-drawing modified after the descriptions of Plachter (1981).

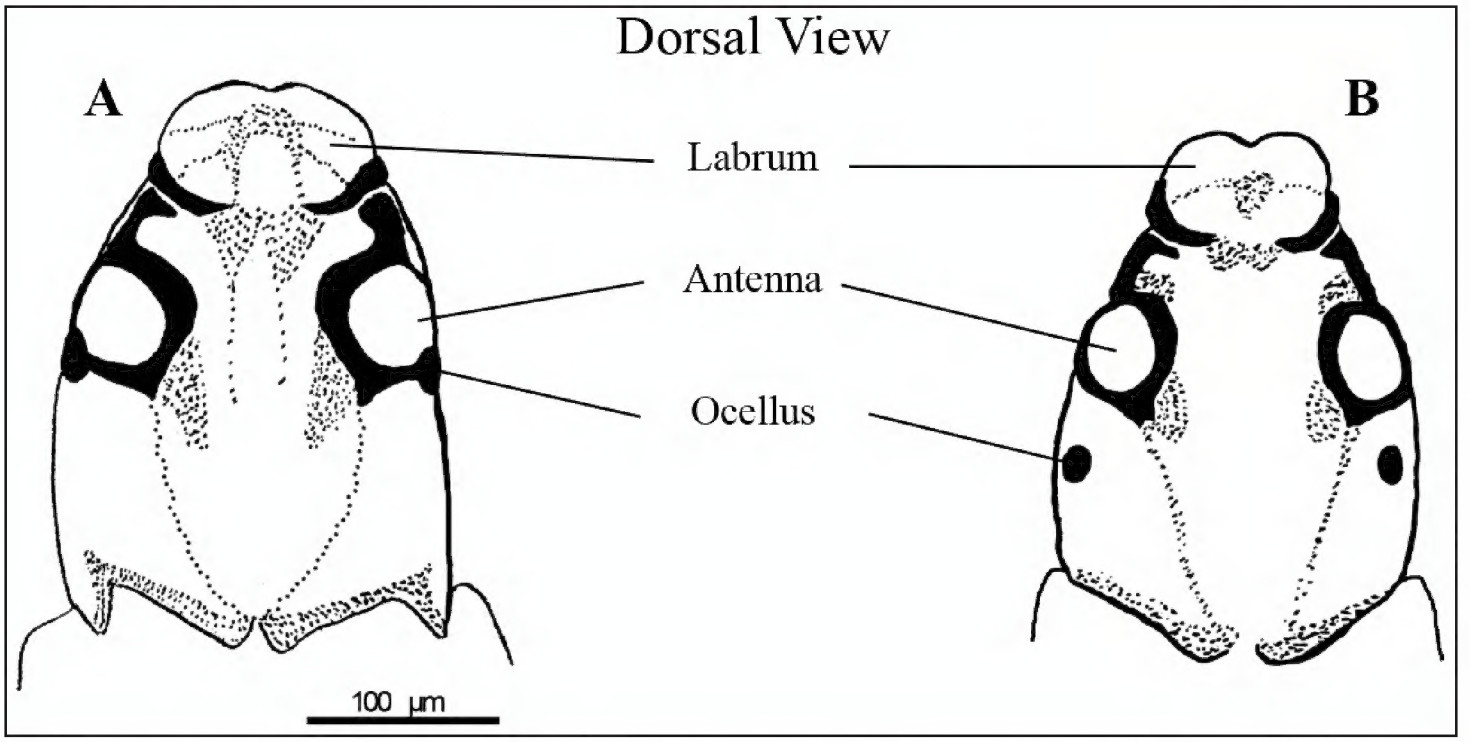

Figure 4. Comparison between two larval stages of *Speolepta leptogaster*. **A** depicts the larger and probably older larva type A which on average is 10 mm long **B** depicts the smaller larva B type which is 5–10 mm long. Dotted regions depict areas of increased pigmentation, black regions illustrate maximum pigmentation.

developmental stages: a) egg: approx. 2 weeks; b) larval stages: 6 – 12 months; c) pupa: approx. 2 weeks and d) imago: 4 – 20 days (Figure 3).

Two types of larvae were present in our material. They can be distinguished by a small deviance of the ocelli position and by body measurements (Figure 4). In type

A, the ocellus is situated adjacent to the antenna whereas in type B, the ocellus is approximately 15 to 20 µm behind the antenna. The head capsule of larvae type B was thinner (A: about 210 µm, B: about 190 µm), slightly shorter (A: about 300 µm, B: about 280 µm) and generally more pointed than in type A. The labrum of larvae type B displays a deeper indentation in the middle of the frontal end. At the proximal end of the head area where the vermiform body begins, the larvae type A displays an edged transition, whereas with larvae type B, it is more semicircular. In the sample set, three larvae were found that appeared as depicted in type B. Two of which had only half the total length of the average length of a normal larva (4.7 mm and 5.0 mm compared to 10 mm). A third specimen was of normal size. Since different larval stages between hatching and pupating are reported in other Mycetophilidae (Madwar 1937), this might explain the morphological discrepancy of larval type A and B in *Speolepta leptogaster*.

Conclusions

Since this work presents the first step towards understanding the dispersal potential of the ecologically versatile species *Speolepta leptogaster* within a small area, subsequent studies should incorporate a larger, pan-European sampling to address diversification patterns for this abundant cave species. Its ecological diversity in congruence with frequent subterranean and sporadic surface animals further challenges the eco-categorical classification system applied for subterranean species.

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References

Baker CH (2010) A new subgenus and five new species of Australian glow-worms (Diptera: Keroplatidae: *Arachnocampa*). Memoirs of the Queensland Museum 55 (1): 11–41.

Clark PU, Dyke AS, Shakun JD, Carlson AE, Clark J, Wohlfahrth B, Mitrovica JX, Hostetler SW, McCabe AM (2009) The Last Glacial Maximum. Science 325 (5941): 710–714. doi: 10.1126/science.1172873

Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9 (10): 1657–1660. doi: 10.1046/j.1365-294x.2000.01020.x

Edwards FW (1925) British fungus-gnats (Diptera,Mycetophilidae). With a revised generic classification of the family. Transactions of the Royal Entomological Society of London 1925: 505–670.

Ersts PJ (2014) Geographic Distance Matrix Generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. http://biodiversityinformatics.amnh.org/open_source/gdmg [accessed on 2014-1-5]

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. Genetics 147 (2): 915–925.

Hamilton-Smith E (1971) The classification of Cavernicoles. National Speleological Society Bulletin 33: 63–66.

Hessisches Landesamt für Umwelt und Geologie: Umweltatlas Hessen - Die Naturräume Hessens und ihre Haupteinheiten http://atlas.umwelt.hessen.de

Hewitt GM (1999) Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society 68: 87–112. doi: 10.1111/j.1095-8312.1999.tb01160.x

Lengersdorf F, Mannheims BB (1951) Das kleine Fliegenbuch: von heimischen Fliegen und Mücken. Reitter 30–33.

Librado P, Rozas J (2009) DNASP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452. doi: 10.1093/bioinformatics/btp187

Li X-Z, Niu C-Y, Huang Q-Y, Lei C-L, Stanley DW (2011) Life cycle of Chetoneura shennonggongensis (Diptera: Keroplatidae: Keroplatinae) from Jiangxi Province, China. Insect Science 16: 351–359.

Madwar S (1937) Biology and Morphology of the immature stages of Mycetophilidae (Diptera: Nematocera). Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 227: 1–110. doi: 10.1098/rstb.1937.0001

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Research 27 (2): 209–220.

Matile L (1962) Morphologie et biologie d'un insecte diptere cavernicole *Speolepta leptogaster* Winnertz (Mycetophilidae). Mémoires du Museum National d'Histoire Naturelle 20: 219–242.

Moseley M (2008) Size matters: scalar phenomena and a proposal for an ecological definition of 'cave'. Cave and Karst Science 35 (3): 89–94.

Petit RJ, Brewer S, Bordács S, Burg K, Cheddadi R, Coart E, Cottrell J, Csaikl UM, van Dam B, Deans JD, Espinel S, Fineschi S, Finkeldey R, Glaz I, Goicoechea PG, Jensen JS, König AO, Lowe AJ, Madsen SF, Mátyás G, Munro RC, Popescu F, Slade D, Tabbener H, de Vries SGM, Ziegenhagen B, de Beaulieu JL, Kremer A (2002) Identification of refugia and post-glacial colonisation routes of European white oaks based on chloroplast DNA and fossil pollen evidence. Forest Ecology and Management 156 (1–3): 49–74. doi: 10.1016/S0378-1127(01)00634-X

Plachter H (1981) Chorionic structures of the eggshells of 15 Fungus- and Root-gnat species (Diptera: Mycetophilidae). International Journal of Insect Morphology and Embryology 10: 43–63. doi: 10.1016/0020-7322(81)90012-X

Racovitza EG (1907) Éssai sur les problèmes biospéologiques (Biospeologica, 1). Archives de Zoologie Experimentale et Génerale, 4e série 6: 371–488.

Reiss M, Steiner H, Zaenker S (2009) The Biospeleological Register of the Hesse Federation for Cave and Karst Research (Germany). Cave and Karst Science 35 (1): 25–34.

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences 74 (12): 5463–5467. doi: 10.1073/pnas.74.12.5463

Schmitz H (1912) Biologisch-anatomische Untersuchungen an einer höhlenbewohnenden Mycetophilidenlarve, *Polylepta leptogaster* Winn. Jaarboek van het Natuurhistorisch Genootschap in Limburg 65–96.

Schönborn W (2003) Lehrbuch der Limnologie. Schweizerbartsche Verlagsbuchhandlung.

Ševčík J, Kjærandsen J, Marshall SA (2012) Revision of *Speolepta* (Diptera: Mycetophilidae), with descriptions of new Nearctic and Oriental species. The Canadian Entomologist 144 (1): 93–107. doi: 10.4039/tce.2012.10

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook A (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequence and compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87: 651–701.

Sket B (2008) Can we agree on an ecological classification of subterranean animals? Journal of Natural History 42 (21–22): 1549–1563. doi: 10.1080/00222930801995762

Skuse FAA (1890) Description of a luminous dipterous insect (Fam. Mycetophilidae), from New Zealand. Proceedings of the Linnean Society of New South Wales (second series) 5: 677–679.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF (1998) Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology 7: 453–464. doi: 10.1046/j.1365-294x.1998.00289.x

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular biology and evolution 28 (10): 2731–2739. doi: 10.1093/molbev/msr121

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123 (3): 585–595.

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132 (2): 619–633.

Vandel A (1965) Biospeleology, the Biology of cavernicolous animals. Pergamon Press, Oxford, 524 pp.

Weber D, Zaenker S, Plassmann E (2007) Pilzmücken in Höhlen und künstlichen Hohlräumen (Diptera: Sciaroidea: Ditomyiidae, Bolitophilidae, Diadocidiidae, Keroplatidae, Mycetophilidae). Entomofauna 28 (11): 125–140.

Weber D (2012) Die Höhlenfauna und -flora des Höhlenkatastergebietes Rheinland-Pfalz/Saarland, 5. Teil. Abhandlungen zur Karst- und Höhlenkunde 36.

Winnertz J (1863) Beitrag zu einer Monografie der Pilzmücken (Mycetophilidae). Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien 13: 637–964.

Zaenker S (2008) Das Biospeläologische Kataster von Hessen. Abhandlungen zur Karst- und Höhlenkunde 32. [Fortschreibung zum 12.04.2008]